IN THE CLAIMS:

1. **(Currently Amended)** An isolated nucleic acid fragment selected from the group consisting of:

(a) an isolated nucleic acid fragment encoding a polypeptide with nitrilase activity as set forth as SEQ ID NO:5;

(b) an isolated nucleic acid molecule that hybridizes with the isolated nucleic acid fragment of (a) under highly stringent hybridization conditions of 6X SSC (1M NaCl), 50~~40 to 45~~ % formamide, 1 % SDS at 37 °C, and a wash in 0.1X~~5X to 1X~~ SSC at 60 to 65~~55 to 60~~ °C; and

(c) an isolated nucleic acid fragment that is completely complementary to (a) or (b).

2. **(Currently Amended)** An isolated nucleic acid fragment comprising a first nucleotide sequence encoding a polypeptide with nitrilase activity having at least 90~~80~~% identity as ~~determined by the Needleman and Wunsch algorithm under default parameters~~ compared to a polypeptide encoded by the sequence identified in SEQ ID NO:5, or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

3. **(Withdrawn)** An isolated nucleic acid fragment encoding a nitrilase enzyme, selected from the group consisting of:

(a) an isolated nucleic acid fragment selected from the group consisting of SEQ ID NO: 4, and SEQ ID NO:16;

(b) an isolated nucleic acid molecule that hybridizes with the isolated nucleic acid fragment of (a) under stringent hybridization conditions of 6X SSC (1M NaCl), 40 to 45% formamide, 1 % SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60 °V' smf

(c) an isolated nucleic acid fragment that is completely complementary to (a) or (b).

4. **(Withdrawn)**An isolated nucleic acid sequence encoding a nitrilase enzyme selected from the group consisting of SEQ ID NO:4, and SEQ ID NO:15.

5. **(Cancelled)**

6. **(Withdrawn)** A polypeptide encoded by the nucleic acid fragments of any of Claims 1, 2, 3, or 4.

7. **(Withdrawn)** A polypeptide according to Claim 6 having the amino acid sequence selected from the group consisting of SEQ ID NO:5 and SEQ ID NO:14.

8. **(Withdrawn)** The polypeptide of Claim 6 further characterized by nitrilase activity on nitrile-containing substrates selected from the group consisting of aliphatic nitriles and aromatic nitriles.

9. **(Previously presented)** A chimeric gene comprising the isolated nucleic acid fragment of Claims 1 or 2 operably linked to suitable regulatory sequences.

10. **(Previously presented)** A plasmid pnitex2 contained in *E. coli* SS1001 having the designation ATCC PTA-1177.

11. **(Original)** An expression cassette comprising the chimeric gene of Claim 9.

12 **(Previously presented)** The expression cassette of Claim 11 comprising plasmid pnitex2.

13. **(Original)** A transformed microorganism comprising the chimeric gene Claim 9.

14. **(Original)** A transformed microorganism comprising the plasmid of Claim 10.

15. **(Original)** A transformed microorganism comprising the expression cassette of Claim 11.

16. **(Original)** The transformed microorganism of Claim 15 wherein the expression cassette is chromosomally integrated.

17. **(Original)** The transformed microorganism of Claim 16 further comprising suitable regulatory sequences.

18. **(Original)** The transformed microorganism of Claim 17 wherein the suitable regulatory sequences comprise

a) at least one promoter selected from the group consisting of the tryptophan operon promoter Ptrp of *E. coli*, a lactose operon promoter Plac of *E. coli*, a Ptac promoter of *E. coli*, a phage lambda right promoter PR, a phage lambda left promoter PL, a T7 promoter, a promoter of the AOX1 gene from *Pichia pastoris*, and a promoter of the GAP gene from *Pichia pastoris,* or is at least one strong promoter selected from the group consisting of *Comamonas*, *Corynebacterium*, *Brevibacterium, Rhodococcus, Azotobacter*, *Citrobacter*, *Enterobacter*, *Clostridium*, *Klebsiella*, *Salmonella, Lactobacillus*, *Aspergillus*, *Saccharomyces*, *Pichia, Zygosaccharomyces*, *Kluyveromyces, Candida*, *Hansenula*, *Dunaliella*, *Debaryomyces*, *Mucor*, *Torulopsis*, *Methylobacteria*, *Bacillus*, *Escherichia*, *Pseudomonas*, *Rhizobium*, and *Streptomyces,* and

b) at least one ribosome binding site from a phage lambda CII gene or selected from the group consisting of ribosome binding sites from a gene of *Comamonas*, *Corynebacterium*, *Brevibacterium, Rhodococcus, Azotobacter* , *Citrobacter*, *Enterobacter*, *Clostridium*, *Klebsiella*, *Salmonella, Lactobacillus*, *Aspergillus*, *Saccharomyces*, *Zygosaccharomyces*, *Pichia, Kluyveromyces, Candida*, *Hansenula*, *Dunaliella*, *Debaryomyces*, *Mucor*, *Torulopsis*, *Methylobacteria*, *Bacillus*, *Escherichia*, *Pseudomonas*, *Rhizobium*, and *Streptomyces.*

19. **(Original)** The transformed microorganism of Claim 18, wherein the host microorganism is selected from the group consisting of *Comamonas*, *Corynebacterium*, *Brevibacterium, Rhodococcus, Azotobacter*, *Citrobacter*, *Enterobacter*, *Clostridium*,

Klebsiella, *Salmonella*, *Lactobacillus*, *Aspergillus*, *Saccharomyces*, *Zygosaccharomyces*, *Pichia*, *Kluyveromyces*, *Candida*, *Hansenula*, *Dunaliella*, *Debaryomyces*, *Mucor*, *Torulopsis*, *Methylobacteria*, *Bacillus*, *Escherichia*, *Pseudomonas*, *Rhizobium*, and *Streptomyces*.

20. **(Previously presented)** A transformed microorganism *E. coli* SS1001 having the designation ATCC PTA-1177.

21. **(Cancelled)**

22. **(Cancelled)**

23. **(Cancelled)**

24. **(Withdrawn)** A method to enzymatically convert nitrile-containing substrates to a carboxylic acid, the method comprising:

(a) contacting, under suitable conditions, a transformed heterologous host expressing the polypeptide of Claim 4 with a nitrile-containing substrate; and

(b) optionally collecting the carboxylic acid produced in step (a).

25. **(Withdrawn)** The method of Claim 24, wherein the nitrile-containing substrate is a dinitrile of the formula NC-R-CN where R is an alkylene group having from 1 to 10 carbon atoms.

26. **(Withdrawn)** The method of Claim 25, wherein the nitrile-containing substrate is 2-methylglutaronitrile.

27. **(Withdrawn)** A method to enzymatically convert nitrile-containing substrate(s) to carboxylic acid(s), the method comprising:

(a) contacting, under suitable conditions, a transformed heterologous host comprising the chimeric gene of Claim 9 with nitrile-containing substrate(s); and

(b) optionally collecting the carboxylic acid produced in step (a).

28. **(Withdrawn)** The method of Claim 27, wherein the nitrile-containing substrate is a dinitrile of the formula NC-R-CN where R is an alkylene group having from 1 to 10 carbon atoms.

29. **(Withdrawn)** The method of Claim 27, wherein the nitrile-containing substrate is 2-methylglutaronitrile.

30. **(Withdrawn)** The method of Claim 27 wherein the suitable regulatory sequences of the chimeric gene comprise an inducible promoter.

31. **(Withdrawn)** The method of Claim 30, the suitable conditions of step (a) further comprising the presence of an inducer of the inducible promoter.

32. **(Withdrawn)** The method of enzymatically converting 2-methylglutaronitrile to the corresponding carboxylic acid, the method comprising:

(a) contacting, under suitable conditions, *E. coli* SW91 designated ATCC PTA-1175 with 2-methylglutaronitrile; and

(b) optionally collecting the carboxylic acid produced in step (a).

33. **(Withdrawn)** An improvement to the process for preparing five-membered ring lactams or six-membered ring lactams from aliphatic α,ω-dinitriles comprising:

(a) contacting an aliphatic α,ω-dinitrile in an aqueous reaction mixture with an enzyme catalyst, whereby the aliphatic α,ω-dinitrile is converted to an ω-cyanocarboxylic acid ammonium salt;

(b) contacting the aqueous product mixture resulting from step (a) with hydrogen and a hydrogenation catalyst, whereby the ω-cyanocarboxylic acid ammonium salt is converted directly to the corresponding lactam without isolation of the intermediate ω-cyanocarboxylic acid, ω-cyanocarboxylic acid ammonium salt, ω-aminocarboxylic acid, or ω-aminocarboxylic acid ammonium salt; and

(c) recovering the lactam from the aqueous product mixture resulting from step (b),

the improvement comprising in step (a) contacting an aliphatic α,ω-dinitrile in an aqueous reaction mixture with an enzyme catalyst selected from the group consisting of

(1) *E. coli* SW91 having the designation ATCC PTA-1175;

(2) *E. coli* DH5a: pnit4 having the designation ATCC PTA-1176;

(3) *E. coli* SS1001 having the designation ATCC PTA-1177; and

(4) *E. coli* SS1002 containing plasmid pnitex2, and

(5) *E. coli* SS1011 containing plasmid pnitex2.

34. **(Withdrawn)** The process of Claim 33 wherein the aliphatic α,ω-dinitrile has the formula

$$NCCX_a(R)(CH_2)_nCN,$$

where a = 0 or 1, where X = hydrogen when a = 1, and R = H, alkyl or substituted alkyl, or alkenyl or substituted alkenyl, or alkylidene or substituted alkylidene, and where n = 1 or 2.

35. **(Withdrawn)** The method of Claim 34 wherein the α,ω-dinitrile is 2-methylglutaronitrile.

36. **(Withdrawn)** The process of Claim 33 wherein the aliphatic α,ω-cyano is unsymmetrically substituted at the α-carbon atom, and the enzyme catalyst is characterized by aliphatic nitrilase activity that produces the ω-cyanocarboxylic acid ammonium salt resulting from regioselective hydrolysis of the ω-cyano group, thereby producing only one of the two possible lactam products during step (b).

37. **(Withdrawn)** The process of Claim 33 further comprising adding, before step (b), ammonium hydroxide, ammonia gas, or methylamine to the aqueous product mixture containing the ω-cyanocarboxylic acid ammonium salt

38. **(Withdrawn)** The process of Claim 37 wherein the amount of ammonium hydroxide, ammonia gas, or methylamine added to the aqueous product mixture is from 0 to 4 molar equivalents relative to the amount of ω-cyanocarboxylic acid ammonium salt present.

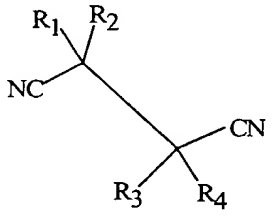
39. **(Withdrawn)** The process of Claim 38 wherein methylamine is added to the aqueous product mixture containing the ω-cyanocarboxylic acid ammonium salt before step (b) and the product of step (b) is an N-methyllactam.

40. **(Withdrawn)** The process of Claim 33 wherein in step (b) the temperature of the aqueous product mixture is from 45 °C to 200 °C.

41. **(Withdrawn)** The process of Claim 33 for preparing five-membered ring lactams or six-membered ring lactams from aliphatic α,ω-dinitriles, wherein:

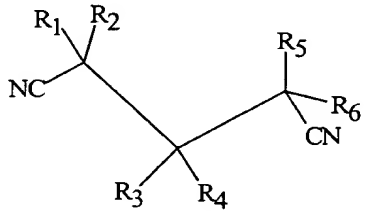
the aliphatic α,ω-dinitrile is of either formula:

I



or

II



where R_1 and R_2 are both H, and

R_3, R_4, R_5, and R_6 are each independently selected from the group consisting of H, alkyl or substituted alkyl, or alkenyl or substituted alkenyl, or R_3 and R_4 taken together are alkylidene or substituted alkylidene, or independently R_5 and R_6 taken together are alkylidene or substituted alkylidene.

42. **(Withdrawn)** The method of Claim 33 or 41 wherein the enzyme catalyst is in the form of whole microbial cells immobilized in or on an insoluble support.

43. **(Withdrawn)** A method for using a native microbial gene encoding a protein characterized by a nitrilase activity on nitrilase-containing substrates to obtain a mutated microbial gene encoding a protein characterized by an increased specific nitrilase activity on nitrile-containing substrates and/or an increased stability of the nitrilase, one or both characteristics increased relative to that of the native microbial gene, the method comprising the steps of

(i) contacting restriction endonucleases with a mixture of nucleotide sequences to yield a mixture of restriction fragments, the mixture of nucleotide sequences comprising

a) a native microbial gene;

b) a first population of nucleotide fragments which will hybridize with the nucleotide sequences of the native microbial gene of (i)(a); and

c) a second population of nucleotide fragments which will not hybridize to the nucleotide sequences of the native microbial gene of (i)(a),

(ii) denaturing the mixture of restriction fragments of step (i);

(iii) incubating the denatured mixture of restriction fragments of step (ii) with a polymerase; and

(iv) repeating steps (i), (ii), and (iii) a sufficient number of times to yield a mutated microbial gene encoding a protein characterized by an increased specific nitrilase activity on nitrile-containing substrates and/or an increased stability of the nitrilase, one or both characteristics increased relative to the nitrilase activity of the native microbial gene.

44. **(Withdrawn)** The method of Claim 43 wherein the native microbial gene is *Acidovorax facilis* 72W and the nitrile-containing substrate is 2-methylglutaronitrile.

45. **(Withdrawn)** A mutated microbial gene encoding a protein characterized by an increased specific nitrilase activity on nitrile-containing substrates and/or an increased stability of the nitrilase, one or both characteristics increased relative to the nitrilase activity of a native microbial gene, the mutated microbial gene produced by the method of Claim 43.

46. **(Original)** The transformed microorganism of Claim 19, wherein the host microorganism is *E. coli* strains MG1655 (ATCC 47076), W3110 (ATCC 27325), MC4100 (ATCC 35695), or W1485 (ATCC 12435).

47. **(Previously presented)** An isolated nucleic acid fragment encoding a polypeptide having the amino acid sequence of SEQ ID NO:5.

48. (Cancelled)

49. **(Currently amended)** An isolated nucleic acid fragment comprising a first nucleotide sequence encoding a polypeptide with nitrilase activity having at least ~~90~~ 95 % identity as ~~determined by the Needleman and Wunsch algorithm under default parameters~~ compared to a polypeptide encoded by the sequence identified in SEQ ID NO:5.